FORMALIN.

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NOTE.—This article was written at the request of the Secretary, the reagent appearing to be of sufficient importance to justify giving our members the benefit of it as soon as possible. It was at first intended merely as an abstract of the article in the American Monthly Microscopical Journal (vol. xv, p. 104), but embodies the result of so much additional experience as to be almost a new paper.

Probably there has been no more important recent accession to the armamentarium of the bacteriologist than formalin, which is the trade name of a 40 per cent. solution of formic aldehyde in water. Formic aldehyde is a well-known gas having the composition CH_2O. It was first successfully prepared in 1867 by Hofmann by passing the vapor of methyl alcohol (wood spirit) over ignited platinum wire.

Attention was first called to the germicidal action of formic aldehyde in 1886 by Low, and further work was done upon the compound by Aronson and by Berlioz and Trillat, but as yet its qualities and uses are not very generally understood or known. My attention was first called to it in October, 1893, and upon trial I was so well pleased with its action that I have since made general use of it, not only as a germicide, but in various other ways, especially as a hardening fluid, fixative and preservative.

Among the advantages of formalin as a germicide may be mentioned its remarkable powers of penetration, its rapid and effective action, the fact that it does not injure fabrics or corrode metallic instruments, and the ease with which it can be applied as a spray, liquid, or gas, being miscible in all proportions with water and alcohol, and readily volatilized even at the ordinary temperature.

As a gas or in strong solution formic aldehyde acts as an irritant to the conjunctiva and air passages, but as vet no unpleasant results have been recorded as having followed its use. The manufacturer states that no bad effects have been observed among the workmen

in the factory, who are constantly exposed to the action of the gas, aside from the slight transitory irritation at first perceived, but he does not recommend the internal use of the solution. In what quantity and to what extent it would prove poisonous if ingested I am unable to state; but to disinfect the hands it can be used in from one-half to one per cent. solution, as a spray in 1 to 500 or stronger solution, as a lotion in 1 to 1,000 or stronger solution, and so on. To small (venereal) ulcers we have applied the pure formalin without any bad effects other than pain and with benefit, but on account of the pain occasioned I think it better to use a more dilute solution of the gas. No harm has followed the accidental contact of the 40 per cent. solution with the uninjured skin, the part having been immediately washed off, but immersion of the hand in a two per cent. solution for half an hour caused some roughness of the skin and slightly impaired the tactile sensibility, these results being observed for a day or two. For disinfecting instruments I use one part in five to ten parts of alcohol or place them for some time in a one or two per cent. aqueous solution.

According to Schering, Berlioz and Trillat found that anthrax bacilli were destroyed by a 1 to 50,000 solution, and Aronson found that a 1 to 20,000 solution prevented the development of the typhus and anthrax bacilli, as well as the staphylococcus pyogenes aureus; also that J. Stahl proved by numerous very elaborate experiments that after one hour's exposure to a one per mille or a quarter of an hour's exposure to a one and one-half per mille solution of formalin the most resistant micro-organisms were destroyed.

In a series of experiments with formalin on the *bacillus diphtheriæ* conducted last fall and winter I found it to be very fatal to germ life and that it possessed a wonderful power of penetration, but I did not find it effective in the extremely dilute solutions above recorded. The conditions, however, may have been quite different, and it must be borne in mind that much depends upon the mode of application of a germicide, the number of the bacteria acted upon, the nature and amount of the associated material, and the length of time that it is allowed to act as to the result. For instance, it will take a much more powerful germicide, acting for a longer time, to effect the destruction of a large number of bacteria growing upon a solid culture medium where several layers are superimposed one upon another and the deeper ones are not only protected by the superficial layers but also by a considerable quantity of the viscid,

gelatinous mass so commonly secreted by bacteria than where a comparatively small number are introduced into an aqueous solution of the germicide. It is for this reason that failure to check the growth of bacteria in the body so often results, it being impossible to reach those beneath the surface with any germicide that can be employed with safety to the individual or diseased part; but herein is conceived to be one of the most important advantages of formalin, as it appears to me to possess a greater power of penetration than any other germicide and to be effective in a form not injurious to the tissues, or, if injurious at all, certainly to a slight extent only.

In my first experiments I inoculated glycerine agar plates by drawing a needle charged with diphtheria bacilli through the medium, and then lightly sprayed the surface with various per cent. solutions of formic aldehyde down to one per mille, and then placed the plates in the incubator at the most favorable temperature for development, but in no case was any growth obtained.

Next glycerine agar plates were made in the usual way and subjected to the same treatment with similar results. Plates inoculated by each of the above methods and exposed to the vapor spontaneously generated (by pouring three or four drops of formalin on a block of kaolin and placing this in the Petri dishes used for the plate cultures) also remained sterile.

Freshly made smear cultures were also subjected to the spray as well as to the vapor generated from three or four drops of formalin placed on the cotton plugs with which the tubes were stopped, and development was prevented in each case. Other smear cultures were made and allowed forty-eight hours in which to obtain an abundant development before applying the spray and vapor, and on the following day the fact of the sterilization was tested by making subcultures, and was found to have been complete.

Stick cultures were also made, and some of them subjected to the vapor generated from formalin on the plug, while into other tubes about five drops of solutions varying in strength from 1 to 100 to 1 in 20,000 were poured. The smear cultures of forty-eight hours' growth proved the ability of even dilute solutions of formic aldehyde, as well as the vapor itself, to penetrate several layers of bacteria and destroy the deeper ones as well as the superficial, and the stick cultures revealed even a greater power of penetration, since the germs introduced by the needle through the solid medium to the depth of an inch or more were inhibited in their development, if

they were not destroyed, although the quantity of the germicide used was scarcely sufficient to cover the surface of the medium.

Solutions up to 1 in 500 were invariably inimical to all bacteria encountered. Diphtheria bacilli were destroyed by weaker solutions up to 1 in 2,000; but the very dilute solutions failed to prevent their development. Mould fungi developed readily from spores falling into the tubes from the air in which solutions of 1 to 2,000 had been applied, and very weak sprays only contaminated the cultures by carrying into the tubes from the air saprophytes, which, not being destroyed, developed in great profusion.

Not only are there great differences in the resistance of different species of bacteria to the action of destructive agents, but, as regards formalin, specific differences seem to exist which may be taken advantage of in the differentiation of allied species—*e. g.*, Schild has discovered that the typhoid bacillus will not develop in bouillon to which formalin has been added in the proportion of 1 to 15,000, while the bacillus coli communi develops in a 1 to 3,000 formalin bouillon.

I should mention that my experiments with formalin on the bacillus of diphtheria were all verified by parallel cultures, and that almost without exception abundant and characteristic development took place in the control cultures.

Hauser has pointed out the fact that cultures of bacteria may be perfectly preserved by formalin without altering their appearance. This is an important item, as it enables one to illustrate lectures by actual cultures without danger, even in case of breakage of the culture dishes, an accident that I have had happen in passing around cultures of virulent germs. It is necessary only to put a few drops of the pure formalin on the cotton plug when the characteristic growth has been obtained and after twenty-four hours they will be found to be sterile. If, now, the tubes are sealed to prevent evaporation, they can be kept until wanted without any perceptible change. Colonies in gelatin or agar plates can be cut out together with a little of the medium, transferred to the slide, covered, some of the fresh medium run under, exposed to formalin vapor for twenty-four hours, and a ring of cement run on, making a permanent preparation; or, as I prefer, a little formalin may be added to a 10 or 20 per cent. solution of gelatin, rendered liquid by heat, and this run under the cover. Treated in either of the above ways the gelatin is rendered insoluble.

Gelatin is not only rendered insoluble by the action of formic aldehyde (in solution or vapor) at the ordinary melting point, but can be placed in the flame of the Bunsen burner, and will char and burn without melting. I have taken advantage of this fact in fixing sections to the slide, and it seems to me that a long-felt want has thus been supplied. It is true that there are already many methods in use, but none of them, so far as my experience goes, are entirely satisfactory. With most, if not all, of them the sections will sometimes slip, but not so with the formalin-gelatin method. The most satisfactory method that I was previously familiar with is the gelatin fixative, which was described some time ago in The Microscope by Dr. Wm. M. Gray, of the Army Medical Museum, but it was not satisfactory to me when I wished to use aqueous stains, as is necessary in staining bacteria, and sometimes desirable in other cases. As modified by me the method is as follows:

A one-half to one per cent. solution of the best French or German gelatin in distilled water is made by the aid of gentle heat and filtered. To this is added a few drops of formalin for each gram of solution. Two or three drops of the formalin-gelatin are placed on a slide and the section floated upon it. Gentle heat is now applied, and as the paraffine softens all the wrinkles will disappear, when the excess of fixative should be drained off and the slide set aside on its end or edge to dry, after which the paraffine may be removed by an essential oil in the usual way, then passed through alcohol and the section stained.

The formalin not only renders the gelatin insoluble, so that the section may be left for hours or even days in water without slipping, but at the same time acts as a preservative for the gelatin solution. Sufficient heat to melt the paraffine must be avoided. As a rule, I think it is better to merely warm the slide a little before putting on the section, remembering that the object of heating is simply to soften the paraffine enough to get rid of wrinkles. Another caution is that sufficient time must be allowed to insure against moisture, as this is incompatible with the clearing agents that must be used. I generally cut the sections one day and stain them on some subsequent day, although they may be attached and stained on the same day if necessary.

During an entire season's work I do not remember to have lost a single section thus attached to the slide. Celloidin as well as paraffine sections may be attached in this manner. As to the effect upon

the staining process I can say from experience that the fixative does not in the least interfere with the application of any of the stains thus far tried, which includes the aniline dyes, toxylin, sulphindigotate of soda, and carmine. I thought at first it did seriously interfere with the action of the carmine dyes, but have since learned that the carmine used in my staining fluids was not good. Fresh tissues may be placed in borax, lithium, alum carmine, or hæmatoxylin to which 5 per cent. of formalin has been added, and stained in bulk and hardened at the same time, thus shortening the process of hardening and staining, the result being all that can be desired.

As a hardening agent I use formalin now almost exclusively and prefer it to Mueller's fluid, alcohol, etc. It hardens very rapidly, most tissues becoming hard enough to cut in twenty-four hours, so far as consistency is concerned, and there is no appreciable shrinkage. If it is wished to imbed in paraffine the tissues must be transferred to alcohol, say 50 or 70 per cent., and finally to absolute, but I do not think that there is the same amount of shrinkage as if hardened from the start in alcohol. I use from 2 to 10 per cent. and sometimes stronger solutions, according to the size of the object, the density of the tissue, and the amount of time I wish to allow. When once fixed, a one per cent. solution is sufficient to preserve the tissue. In the case of soft or spongy tissue (brain or lungs) I use a stronger solution than for denser tissues, such as the liver or spleen.

Formalin is said to not coagulate albumen, differing in this respect from corrosive sublimate, which combines with the albumen to form an insoluble albuminate, rendering it inert. I at first adopted this theory or statement without question, but I have since asked myself the question, Upon what does its power to fix tissues depend, if not upon the property of coagulating albumen? I use it, however, to preserve albuminous urines, which it does without precipitation of the albumen or alteration of the sediment, one exception only having been observed. If it does coagulate albumen, however, it seems to have the power of penetrating the coagulated mass and destroying bacteria.

As to the use of formalin in surgery and as to the clinical aspects of the subject, it is not within the scope of this article to go.